PHAGOCYTOSIS OF RED CORPUSCLES.*

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SAVTCHENKO,[1] Levaditi,[2] Gruber[3] all observed that injection of serum of rabbits immunized with guinea-pig blood into the peritoneal cavity of guinea-pigs is followed by a marked erythrophagocytosis not only in the abdomen, but also in the blood-making organs, especially the spleen, and even in the circulating blood (Levaditi). In this phagocytosis take part microcytes as well as macrocytes. Ruziczka noted the occurrence of phagocytosis *in vitro* of red corpuscles in the presence of the corresponding immune serum.[4] This phagocytosis was ascribed by Savtchenko to the action upon either the phagocytes or the erythrocytes of the amboceptor or *substance sensibilisatrice* in the immune serum, and this explanation is not contested by either Levaditi or Gruber. A year ago Neufeld and Töpfer[5] showed that the blood of rabbits immunized with goat blood contains a substance that by action on goat corpuscles after absorption by them renders them subject to phagocytosis by guinea-pig leucocytes *in vitro*. This substance, which they designated as hemotropic, had no direct action on the leucocytes. Elsewhere[6] I have pointed out that such substances should be called opsonins (hemopsonins) because of their analogy to the bacteriopsonins of Wright and Douglas who were the first to show clearly the opsonic function of the serum in phagocytosis of bacteria. Barratt[7] found that doves immunized with hen blood give a serum that is strongly opsonic for hen corpuscles and demonstrated that in this, as well as other cases, the immune serum acts upon the erythrocytes and not directly upon the phagocytes. Neufeld and Töpfer, as well as Barratt conclude that the hemotropic substances, or hemopsonins, are distinct

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[1] *Ann. de l'Inst. Past.*, 1902, 16, p. 106.

[2] *Ibid.*, p. 233.

[3] *Wien. klin. Wchnschr.*, 1903, 16, p. 1097.

[4] See Gruber, *loc. cit.*

[5] *Centralbl. f. Bakt.*, 1905, Orig. 37, p. 456.

[6] *Jour. Am. Med. Assoc.*, 1906, 46, p. 1407.

[7] *Proc. Roy. Soc.*, B, 1905, 76, p. 524.

from the amboceptors and agglutinins, because a serum may be lytic or agglutinating, but not opsonic, and vice versa, with respect to certain erythrocytes; and recently I have shown that the specific amboceptors in an immune serum (serum of rabbits immune to goat blood) may be separated from the specific opsonins by absorption methods.[1] Keith[2] also has shown that hemopsonins are distinct from hemolytic amboceptors.

Barratt found immune opsonins to be quite resistant to heat and I have observed that heating to 70°C. for one hour the serum (diluted by one-half with m/8 NaCl solution) of rabbits immune to goat blood had no pronounced effect upon its opsonic power. Immune sera retain their hemopsonic power for months when kept at 3°–4° above 0° C.

TECHNIQUE.

Phagocytosis of red corpuscles *in vitro* may be studied in the following manner:

In case the serum in question is lytic for the corpuscles employed, which is commonly the case with immune serum, i. e., serum of an animal injected several times with increasing doses of alien blood,* then it is necessary first to destroy the hemolytic complement by heating at say 60° C. for 30 minutes in order to prevent laking. So far as my experience goes heating in this manner has no appreciable effect upon the hemopsonins. A small quantity of serum, e. g., 0.1 c.c. is now mixed with 0.1 or 0.2 c.c. of a 5 per cent suspension in NaCl solution of washed red corpuscles, and to this is added a small quantity of washed leucocytes contained in citrated or defibrinated blood or obtained from fresh aleuronat exudates, and the mixture placed at 37° C. for 60 minutes or so when smears are made and stained. When the serum, the erythrocytes, and the leucocytes employed are homologous the heating of the serum is unnecessary. If the serum is actively opsonic for the erythrocytes and the leucocytes phagocytically active, many of these will be found to be packed with unchanged red corpuscles. Laking in the ordinary sense does not occur, but after some time the intraleucocytic corpuscles may swell up or coalesce to form quite large globules. Further than this I have been unable to trace the fate of the corpuscles ingested by leucocytes *in vitro*. In many instances leucocytes of various species may be used as phagocytes, and one is not necessarily limited for this purpose to the leucocytes of the species furnishing the serum. Thus the serum of rabbits immune to goat blood may subject corpuscles to phagocytosis by dog, guinea-pig, human, and other leucocytes.

[1] *Jour. Infect. Dis.*, 1906, 3, p. 434. [2] *Proc. Roy. Soc.*, B., 1906, 77, p. 537.

* In rabbits immune hemopsonins develop in response to injections of goat serum, goat corpuscles washed repeatedly in NaCl solution, and the washed stroma of laked corpuscles.

THE SPECIFICNESS OF IMMUNE HEMOPSONINS.

The extent to which immune serum is specific in its hemopsonic powers does not seem to have received much consideration as yet. Naturally one would expect to find that immune hemopsonin like the hemolytic amboceptor and hemagglutinins may find suitable receptors in other corpuscles than the particular kind used for immunization. Indeed, experiments with serum of rabbits immunized to goat corpuscles shows this supposition to be correct: 0.1 c.c. of this serum (heated to 60° C. for 30 minutes) has been found to opsonize, for phagocytosis by washed dog leucocytes, not only goat corpuscles, but also the corpuscles of sheep, dog, rabbit, chicken, pigeon, guinea-pig, and man. Using guinea-pig leucocytes as phagocytes the range of opsonic action in the same quantity of immune serum is limited almost exclusively to goat and sheep corpuscles. Using falling quantities of the immune serum and dog leucocytes as phagocytes it is found that the opsonic effect becomes limited to goat and sheep corpuscles, the approximate minimum opsonic dose for which has been found to be 0.0125 c.c. under the conditions of these experiments. These facts are set forth in Table 1.

TABLE 1.

PHAGOCYTOSIS OF DIVERSE CORPUSCLES UNDER THE INFLUENCE OF SERUM OF RABBITS, (1) IMMUNE TO GOAT BLOOD AND (2) NORMAL.

Serum + m/8 NaCl Solution, 5 Per Cent Suspension Washed Corpuscles, Sus. Washed Leucocytes of Dog, Equal Parts	PHAGOCYTOSIS		
	Immune Serum		Normal Serum
	0.1c.c.	0.0125.c.c.	0.1c.c.
Goat corpuscles + dog leucocytes	+++	++	0
Sheep " + " "	+++	+	0
Dog " + " "	+++	0	0
Guinea-pig " + " "	+++	0	0
Rabbit " + " "	++	0	0
Human " + " "	++	0	±
Chicken " + " "	++	0	0
Pigeon " + " "	+	0	0
Beef " + " "	0	0	0
Goat " + Guinea-pig leucocytes	++	+	0
Sheep " + " " "	+	0	0
Dog " + " " "	0	0	0
Guinea-pig " + " " "	0	0	0
Rabbit " + " " "	0	0	0
Human " + " " "	0	0	0
Chicken " + " " "	0	0	0
Pigeon " + " " "	0	0	0
Beef " + " " "	0	0	0

+++=Phagocytosis well marked. +=Phagocytosis good.
++= " marked. ±= " trace.

Here it is shown also that normal rabbit serum has but little hemopsonic effect. The hemopsonic power of normal rabbit as well as of other sera appears to vary considerably, however, and the susceptibility of erythrocytes of various animals to opsonification is also a variable quality. At all events the amount of hemopsonin in normal serum would seem to be much smaller than that of bacteriopsonin.[1] Barratt found that normal sera may contain erythrocytic opsonins in small amounts.

The results obtained in similar experiments with other immune hemopsonins are shown in Table 2. It is of course possible that

TABLE 2.

RANGE OF OPSONIC POWER OF IMMUNE HEMSOPONINS.

Serum + m/8 NaCl Sol. 5% Suspension Washed Corpuscles, Suspension Washed Leucocytes (Dog) Equal Parts	PHAGOCYTOSIS							
	Goat Serum				Guinea-Pig Serum		Rabbit Serum	
	Immune to Sheep Blood			Normal	Immune to Rabbit Blood	Normal	Immune to Beef Blood	Normal
	0.1c.c.	0.025c.c.	0.006c.c.	0.1c.c.	0.1c.c.	0.1c.c.	0.1c.c. and 0.025c.c.	0.1c.c.
Goat corpuscles.....	++	+	0	0	0	0	++	0
Sheep "	+++	+++	+	0	0	0	0	0
Dog "	0	0	0	0	0	0	0	0
Rabbit "	Tr*	0	0	0	++	0	0	0
Guinea-pig "	Tr*	0	0	0	0	0	0	0
Human "	Tr*	0	0	0	0	0	0	0
Beef "	+	0	0	0	0	0	+++	0

*Tr = trace.

larger quantities of serum or more potent serum might be found to contain opsonins for other corpuscles than those that are opsonized by these sera. Variable susceptibility of the erythrocytes as well as variation in the phagocytic activity of the leucocytes employed may also cause some variation in the results, but in nearly all the sera tested the opsonic effect on dilution becomes limited quite strictly to the corpuscles used for immunization.

The question now arises whether the immune sera that opsonize several kinds of corpuscles do this by virtue of a single opsonic sub-

[1] In connection with the question of the specificity of hemopsonins it is of interest to note that Bulloch and Western (*Proc. Roy. Soc.*, B, 1906, 77, p. 531) have found that opsonins for staphylococci and tubercle bacilli in the serum of normal and vaccinated human beings are distinct substances.

stance or several such. Certain absorption experiments that I have made show that while the corpuscles used for immunization remove all the opsonins in the serum, other corpuscles that are opsonized by the serum may absorb only those opsonins that appear to be peculiar to themselves. Table 3 shows the results of experiments

TABLE 3.

SEPARATION OF SPECIFIC AND COMMON HEMOPSONINS IN IMMUNE SERA.

ABSORPTION OF OPSONIN IN SERUM OF GOAT IMMUNE TO SHEEP BLOOD BY (1) GOAT CORPUSCLES AND (2) SHEEP CORPUSCLES.

1.

Immune serum 0.05+10 per cent suspension washed goat corpuscles 3 c.c. centrifugate.	After one hour at 37° C.
Supernatant fluid 1.5 (0.025 serum)+5 per cent susp. sheep corpuscles 0.2+susp. dog leucocytes 0.2	=Phagocytosis
Supernatant fluid 1.5 (0.025 serum)+5 per cent susp. sheep corpuscles 0.2+susp. goat leucocytes 0.2	=No "
Immune serum 0.025 +5 per cent susp. sheep corpuscles 0.2 + susp. goat leucocytes 0.2	=Phagocytosis

2.

Immune serum 0.05+10 per cent suspension washed sheep corpuscles 3.3.	After one hour centrifugate
Supernatant fluid 1.5 (0.025 immune serum)+5 per cent washed corpuscles goat 0.2+dog leucocytes 0.2 . . .	=No phagocytosis
Supernatant fluid 1.5 (0.025 immune serum)+5 per cent washed sheep corpuscles 0.2+dog leucocytes 0.2 . .	=Trace "
Immune serum 0.025+5 per cent washed sheep corpuscles 0.2+dog leucocytes 0.2	=Phagocytosis

ABSORPTION OF OPSONIN IN SERUM OF RABBITS IMMUNE TO GOAT BLOOD BY (1) SHEEP CORPUSCLES AND (2) GOAT CORPUSCLES.

1.

Immune serum 0.05+5 per cent suspension of washed sheep corpuscles 2.4 c.c.	Centrifugate after one hour at 37° C.
Supernatant fluid 0.6 (0.0125 of serum)+5 per cent susp. goat corpuscles 0.2+suspension dog leucocytes 0.2 . . .	=Good phagocytosis
Supernatant fluid 0.6 (0.0125 of serum) + 5 per cent susp. sheep corpuscles 0.2+suspension dog leucocytes 0.2 .	=No "
Immune serum 0.0125 in NaCl sol. 0.6+5 per cent susp. sheep corpuscles 0.2+suspension dog leucocytes 0.2 .	=Good "

2.

Immune serum 0.05+5 per cent suspension of washed goat corpuscles 2.4 c.c.	Centrifugate after one hour at 37° C.
Supernatant fluid 0.6 (0.0125 of serum)+5 per cent susp. sheep corpuscles 0.2+suspension dog leucocytes 0.2 .	=No phagocytosis
Supernatant fluid 0.6 (0.0125 of serum) +5 per cent susp. goat corpuscles 0.2+suspension dog leucocytes 0.2 . .	=No "
Immune serum 0.0125 in NaCl sol. 0.6+5 per cent susp. goat corpuscles 0.2+suspension dog leucocytes 0.2 . .	=Marked "

of this kind with the serum of goats immune to sheep blood, and with the serum of rabbits immune to goat blood. Similar results have

been obtained with the serum of rabbits immune to beef blood. These experiments warrant the conclusion that immune hemopsonic serum may contain specific and non-specific or common opsonins in every way analogous to the specific and common or group agglutinins,[1] and precipitins that develop in animals immunized with the proper antigens. Whether blood relationship can be brought out by means of immune hemopsonins after the manner that Nuttall has shown serum-precipitins do would seem an interesting problem.

PHAGOCYTOSIS OF HUMAN ERYTHROCYTES BY HUMAN LEUCOCYTES.

The often extensive phagocytosis of red corpuscles and of other cells, especially in the spleen, the marrow, the lymph and hemolymph nodes in various infectious, toxic, and anemic processes, suggested that a study of phagocytosis of human erythrocytes by human leucocytes *in vitro* from the point of view of opsonification might be of interest. For this purpose small quantities of citrated human blood from various sources are washed three or four times in liberal quantities of NaCl solution by means of centrifugalization; in order to secure as many leucocytes as possible the superficial layers of the sedimented blood corpuscles, the "blood cream," are made into a thick suspension in salt solution, and 0.1 or 0.2 c.c. of this suspension is mixed with an equal quantity of the serum, the opsonic effect of which is to be determined; this mixture is then placed at 37° C. for 40 minutes when smears are made and stained.

A brief summary of the essential facts obtained is displayed in Table 4. In no case so far has phagocytosis been observed in mixtures of washed fresh blood cream and salt solution only. Serum is essential for phagocytosis here also. Erythrocytes may take up the active substance—hemopsonin—from the serum and in so doing they become susceptible to phagocytosis by washed leucocytes after every trace of serum has been removed by repeated washing. If sufficient erythrocytes of the proper kind are added, all the opsonin may be removed from the serum.

As shown in Table 4 the sera of patients with various infections and occasionally also the sera of normal persons may be agglutinating

[1] See William Hallock Park, *Jour. Infect. Dis.*, 1906, Suppl. No. 2. p. 1; also Park and Collins, *Jour. Med. Res.*, 1901, 7, N. S., p. 401.

or opsonic, most commonly both, for human erythrocytes, especially, it would seem, those from patients with certain diseases, e. g., pneumonia. Phagocytosis without agglutination may occur, but that is rare; agglutination without phagocytosis is more common; agglutination and phagocytosis most common if the serum has any action whatsoever upon the red corpuscles used. This phagocytic and agglutinative property of certain sera, especially, so far as my experience goes, of some typhoid patients appears to be lost very slowly on standing (ice-chest). In other sera it may be lost more quickly.

TABLE 4.

OPSONINS AND AGGLUTININS FOR HUMAN CORPUSCLES IN HUMAN SERA.

WASHED BLOOD CREAM + SERUM EQUAL PARTS	PHAGOCYTOSIS AND AGGLUTINATION BY VARIOUS SERA													
	Typhoid F. 1		Scarlet F.		TyphoidF.2		Erysipelas		Pneumonia		Normal		Normal	
	Aggl.	Phg.	Aggl.	Phg	Aggl.	Phg	Aggl.	Phg.	Aggl.	Phg.	Aggl	Phg	Aggl	Phg
Pneumonic blood cream	+++	+++	+++	++	+	++	++	++	o	+++				
Typhoid blood cream...	+++	+	o	o			++	±						
Scarlet F. blood cream..	+++	+++	+++	+	++	+								
Normal blood cream....			+	o	++	+	+	o	o	o				
*Erysipelas blood cream					++	+	*++	*+						
*Pneumonic blood cream					+++	++	++	+	*++	*+				
Rheumatic blood cream			o	o			o	o			o	o		
Cellulitis blood cream...			o	o			o	o			o	o		
Normal blood cream....			+	o	+	+	+	o	o	o	o	o		
Normal blood cream....			++	+							++	+		
Pneumonic blood cream			++	++									++	++
Normal blood cream....			o	o									o	o

+++ = phagocytosis well marked. ++ = phagocytosis marked. + = phagocytosis present.

*In these cases it concerns serum and blood cream from the same person (autophagocytosis autoagglutination).

As indicated the same serum may be opsonic and agglutinating for certain corpuscles but not for others, showing clearly that the condition of the corpuscles may vary greatly. Whether this variation (which I have noticed even in the corpuscles of the same person at different times) may depend on the lack of suitable receptors or upon resistance to the functional group of the agglutinin or opsonin must be determined by further study. As regards the opsonification.

I have found that the same corpuscles may be sensitized for phagocytosis by leucocytes of one blood but not by those of another, indicating that the state of the leucocyte also may vary. It is too early to make any definite statements as to the erythrophagocytic power of leucocytes and to the susceptibility to opsonification of erythrocytes in different diseases, but I have been struck particularly with the readiness with which phagocytosis (and agglutination) is obtainable in pneumonic blood cream and with the opsonic power of certain typhoid sera. On the whole my observations as to hemagglutinins in human serum correspond with those previously recorded by Eisenberg,[1] Herter and others.[2]

So far I have not encountered any instance of lysis of human red cells by human serum.

As shown in the table the serum of an individual may be agglutinative for the corpuscles of that individual and also subject them to phagocytosis by their fellow leucocytes. In other words, serum may be auto-agglutinative and auto-opsonic. My colleagues, Dr. Rosenow and Dr. Davis, on several occasions have noted autophagocytosis in defibrinated blood of patients with pneumonia and with acute (meningococcus) meningitis. Wright[3] observed phagocytosis of red cells in two cases of pneumococcus infection (cellulitis, cystitis). He suggests that this may be due to toxic damage to the red cells which then are taken up without the intervention of opsonin. The extent to which this explanation will hold remains to be seen. We know that phagocytosis of some objects—certain bacteria, coal particles, carmin granules—may occur without the presence of serum and consequently presumptively without opsonin. Hence it is possible that altered red cells may be taken up without opsonification and the exact mechanism of erythrophagocytosis under different conditions merits careful consideration. There can be no question, however, that in certain diseases, notably typhoid fever, the serum acquires the power to promote phagocytosis by its opsonic action upon the red cells. I have not been able to demonstrate the presence of hemopsonins in cultures of typhoid bacilli, and it would seem that the hemopsonin in the serum of typhoid patients develops as the result of reactions

[1] *Wien kl. Wchnschr.*, 1901, 14, p. 1021.
[2] *Medical Record*, 1902, 61, p. 117.
[3] *Brit. Med. Jour.*, 1906, Jan. 20, p. 143.

in the infected body. Mallory[1] suggested that the phagocytosis of erythrocytes and other cells in typhoid fever is due to stimulation of phagocytes by bacterial toxins. Naturally the demonstration of hemopsonins will lead to special stress being placed on the importance of opsonification in infectious erythrophagocytosis.

As already mentioned Savtchenko, Levaditi, and Gruber showed that the injection of the serum of rabbits immunized with guinea-pig blood into the peritoneal cavity of guinea-pigs is followed by marked erythrophagocytosis in the peritoneum as well as in the blood-making organs, especially the spleen and also in the blood (Levaditi). Portis[2] observed that in dogs injected with the serum of goats, immunized with dog thyroid, there occurred an extensive phagocytosis of red corpuscles by hyaline and endothelial cells.[3] The occurrence of phagocytosis of red cells *in vivo* under the influence of sera containing specific hemopsonins consequently appears well established. On the whole it seems reasonable that the development of hemopsonins and consequent phagocytosis, in some degree at least, will serve to explain the anemia of infections in which erythrophagocytosis occurs.

On injection of immune serum Stschastnyi[4] noted, in the first place, phagocytosis of red cells, and later, so he believes, the gradual transformation of the phagocytic leucocytes into typical eosinophils, the granules of which he derives from hemoglobin. Whether, in case this view of the nature of eosinophils becomes general, hemopsonins play any part in their formation under normal and pathological conditions, e. g., after infections, must be left to future study. Paroxysmal hemoglobinuria as well as other destructive blood diseases invite investigation from the point of view of hemopsonins. Eason[5] describes phagocytosis of normal red corpuscles (human) by

[1] *Jour. Exp. Med.*, 1898, 3, p. 611, and 1900, 5. p. 1.

[2] *Jour. Infect. Dis.*, 1904, 1, p. 127.

[3] In connection with this observation it may be worth while to record the results of a small series of experiments with cytotoxic sera. At my request Dr. S. P. Beebe of New York kindly sent me sera of Belgian hares injected with nucleoproteids of the adrenal and the kidney of the dog and of the thyroid of a fatal case of exophthalmic goiter. Of these sera the anti-adrenal had no opsonic effect upon beef, sheep, goat, dog, rabbit, or human corpuscles, using dog leucocytes as phagocytes. The anti-renal had only slight effect on goat and sheep corpuscles. The anti-thyroid contained traces of opsonin for goat, sheep, dog, rabbit, and guinea-pig corpuscles and opsonin in large quantities for human erythrocytes.

[4] *Ziegler's Beiträge*, 1905, 38, p. 456.

[5] *Edin. Med. Jour.*, 1906, 19, N. S., p. 43.

normal leucocytes in the presence of the serum of hemoglobinuric patients. And we know that in pernicious anemia there is phagocytosis of red cells.

SUMMARY.

Normal serum may contain opsonins for heterologous, and in some instances also (human serum) for homologous, erythrocytes.

Repeated injections with alien blood commonly give rise to the accumulation of hemopsonins in the blood.

Immune hemopsonic serum may contain common or non-specific hemopsonins as well as specific hemopsonins directed particularly against the corpuscles of the blood employed for the injection.

Immune hemopsonins possess a high degree of resistance to heat and other influences.

Hemopsonins render red corpuscles susceptible to phagocytosis by various leucocytes, including the homologous, but the phagocytic activity of different leucocytes toward opsonized erythrocytes may vary.

Human serum may contain agglutinin and opsonin for human erythrocytes. This appears to be the case especially in typhoid fever. The corpuscles of various individuals vary in their susceptibility to agglutination and phagocytosis by human leucocytes and the phagocytic power of leucocytes may vary. Auto-agglutinins and auto-opsonins appear to occur.

The demonstration of opsonins for human corpuscles in human serum may help us to a better understanding of the phagocytosis of erythrocytes in infectious and other processes.